REMARKS

Claims 38, 40, 54-56, 63, 64, 66, 72, 74, 80-85, 88 and 100-105 are pending in this application. This Amendment will cancel claims 38, 40, 54-56, and 100-102. Applicant expressly reserves the right to pursue protection of any canceled subject matter in a continuing application. This Amendment also will amend claims 63 and 74, and add claim 106. The claim 74 amendment is supported, at least, at page 10, lines 5-6; page 24, lines 14-15; page 24, line 17; and page 24, lines 30-32. New claim 106 merely combines the features of claims 63, 74, and 103 in a single claim. No new matter is introduced by any of the foregoing claim amendments, and no new issues are raised.

Entry of the amendments after final action is appropriate because the amendments are believed to place the claims in a condition for allowance. Moreover, entry of the amendment would reduce the number of claims, remove the canceled subject matter from consideration and, thereby, simplify issues for appeal.

Upon entry of the foregoing claim amendments, **claims 63, 64, 66, 72, 74, 80-85, 88, and 103-106 will be pending in this application**. Consideration of the claim amendments herein and reconsideration of the provisional double patenting, 35 U.S.C. §112, first paragraph (written description), and 35 U.S.C. §102(a) rejections is requested.

Obviousness-Type Double Patenting:

Claims 63, 64, 66, 73, 74, and 87 have been "provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3 and 9-12 of copending Application No. 10/783,672." Without conceding to the accuracy of this rejection, U.S. Patent Application No. 10/783,672 has been abandoned as evidenced by a Notice of Abandonment, dated September 6, 2006, which notice is available on public PAIR. In view of the abandonment of U.S. Patent Application No. 10/783,672, there is no issue of double patenting with respect to that application, and Applicant respectfully requests that this rejection be withdrawn.

Claim Rejections under 35 U.S.C. §112, first paragraph:

Claims 38, 40, 54-56, 63, 64, 66, 72, 74, 80-85, 88 and 100-105 have been rejected under 35 U.S.C. §112, first paragraph (written description) on the bases that (i) "the limitation . . . 'immunoprecipitates with the (a) SWI/SNF chromatin-remodeling complex'" in claims 38 and 63, allegedly, is new matter; and (ii) "the limitation . . . 'SWI/SNF chromatin remodeling complex consisting of BRG1 and BAF155'" in claim 38, allegedly, is new matter.

Applicant respectfully submits that appropriate and sufficient support in the specification previously was provided for the alleged new matter in each of the rejected claims and, at least for this reason, traverse this rejection. Nevertheless, to facilitate prosecution of this application, claims 38, 40, 54-56, and 100-102 will be canceled by this Amendment. Thus, this rejection is moot and should be withdrawn as to claims 38, 40, 54-56, and 100-102. The only portion of this rejection that will not be moot after entry of this Amendment is the rejection of claims 63, 64, 66, 72, 74, 80-85, 88 and 103-105 on the basis that "the limitation . . . 'immunoprecipitates with the (a) SWI/SNF chromatin-remodeling complex'" in claim 63, allegedly, is new matter.

To facilitate prosecution of this application, claim 63 has been amended to remove the allegedly new matter. Amended claim 63 recites, in relevant part, that the ". . . zinc finger DNA binding domain peptide *interacts directly* with a SWI/SNF chromatin remodeling complex . . . " (emphasis added). The specification clearly supports "zinc finger DNA binding domain peptide[s] that *interact[] directly* with a SWI/SNF chromatin remodeling complex." For example, page 9, line 27 through page 10, line 9 states, in relevant part (emphasis added):

> . . . SWI/SNF *interacts directly* with a particular class of transcription factors that contain zinc finger DNA-binding domains. . . . Interaction occurs through the *DNA-binding domain* . . . . [T]he inventors have defined the [SWI/SNF] subunits that interact directly with zinc finger DNA-binding domains . . . . Moreover, . . . [SWI/SNF subunits] plus the zinc finger domain *alone* are sufficient to "target"chromatin remodeling to specific promoters.

Further support is found, at least, at page 18, lines 19-21, which states, in relevant part (emphasis added):

> The DNA-binding domains of several zinc finger proteins . . . *interact directly* with SWI/SNF complexes to generate DNase I hypersensitive sites within . . . chromatin.

Finally, the limitation that the "zinc finger DNA binding domain peptide interacts directly with a SWI/SNF chromatin remodeling complex" is presently contemplated by claim 63; for example, in the claim language: ". . . under conditions that permit the direct interaction of the SWI/SNF chromatin remodeling complex and the zinc finger DNA binding domain peptide . . . ." Thus, this feature of amended claim 63 previously has been accepted by the Office and Applicant respectfully submits it is not new matter.

In view of the foregoing arguments and amendments, Applicant respectfully requests that this rejection be withdrawn.

Claim Rejection under 35 U.S.C. §102(b):

Claims 38, 40, 54, 63, 64, 66, 72, 74, 80, 83, 84, 87, 88 and 100-105 have been rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Armstrong *et al*, *Cell*, 95:93-104, 1998 ("Armstrong"). Applicant traverses this rejection, at least, for reasons previously of record and for reasons discussed below.

Upon entry of this Amendment, this rejection will be moot with regard to claims 38, 40, 54, and 100-102, which are canceled herein.

With regard to claims 63, 64, 66, 72, 74, 80, 83, 84, 87, 88 and 103-105, Applicant respectfully requests that the Office reconsider its position. Armstrong does not describe (either expressly or inherently) "*each and every element* as set forth in the claim[s]" (MPEP §2131; emphasis added). In particular, Armstrong does not teach or suggest a method involving "a zinc finger DNA binding domain peptide." Thus, Armstrong can not and does not anticipate the claims under 35 U.S.C. §102(a).

The Office action contends that "Armstrong does teach a zinc-finger DNA binding peptide by teaching use of [full-length] ELKF as there is no[] size requirement engendered by the use of the term 'peptide'" (page 7 of the Office action). As discussed below in more detail, Applicant respectfully submits that this interpretation is not the "plain meaning" of the complete claim term "a zinc finger DNA binding domain peptide."

"[D]uring examination the USPTO must give claims their broadest reasonable interpretation[; t]his means that the words of the claim must be given their plain meaning . . . ." (MPEP §2111.0(I); emphasis added). According to the MPEP, the "plain meaning" of a claim feature must be judged by (i) "*the use of the words in the context of the written description*"; and (ii) as *"customarily [used] by those skilled in the relevant art* (MPEP §2111.0(II) citing *Ferguson Beauregard/Logic Controls v. Mega Systems*, 350 F.3d 1327, 1338, 69 USPQ2d 1001, 1009 (Fed. Cir. 2003); emphasis added).

In contrast to the Office's interpretation, the "context of the written description" makes clear that "a zinc finger DNA binding domain peptide" does not mean "protein" or even any other part of a protein except the zinc-finger DNA-binding domain. For example, in describing one embodiment of the disclosure, the specification states (at page 29, line 27 through page 30, line 4):

> [G]enes that are inactive due to the inability of their regulatory proteins to interact with their DNA binding sites in chromatin, can be activated by *small protein domains (zinc fingers* . . .) that can direct or target chromatin remodeling or modifying complexes to specific DNA sequences . . . . *This avoids the problems with introducing entire proteins . . . into cells* . . . since *small peptide*s can potentially be used.

This passage makes clear that "entire proteins" can be problematic in the context of targeting chromatin remodeling complexes to specific DNA sequences and one solution provided by the disclosure is the provision of small zinc finger domain peptides. By contrasting a zinc finger domain peptide to an entire protein, the specification clearly gives separate meaning to these two terms.

Other passages of the specification also describe the zinc finger domain or motif as separate and clearly distinguishable (*e.g.*, as a stand-alone peptide) from a full-length protein. For instance:

1. Page 10, lines 5-10 describes the inventors' findings with regard to "a full length zinc finger containing protein, EKLF" and "the zinc finger domain alone";
2. Page 22, lines 4-6 explains that an isolated "zinc finger DNA-binding domain was as active as full-length EKLF in generating DNase I hypersensitive site formation in the presence of ERC1";
3. Page 23, lines 9-13 distinguishes "full-length" proteins (*i.e.*, EKLF and GATA-1) from their isolated "zinc finger DNA-binding domains" as components of a GST pulldown assay;
4. The paragraph beginning on page 27, line 3 describes a particular step in an exemplary method for "high-throughput drug screening" involving "multi-well plates that have been coated with a specific concentration of the full length protein or its zinc finger motif."

"[I]n the context of the written description" alone, the plain meaning of "a zinc finger DNA binding domain peptide" clearly means less than a full-length protein; otherwise, the distinctions between zinc finger peptides and full-length proteins containing zinc finger domains made throughout the specification would be meaningless.

Moreover, the customary usage by those skilled in the relevant art makes clear that the "plain meaning" of "a zinc finger DNA binding domain peptide" does not mean "protein" or even any other part of a protein except the zinc-finger DNA-binding domain. At the time of the invention, proteins containing zinc-finger DNA binding domains were known (*e.g.*, specification at page 9, lines 8-23). It was further known that zinc-finger DNA binding domains are distinct modules that are *structurally and functionally independent* of the protein containing them (*e.g.*, Klug, *J. Mol. Biol.*, 293:215-218, 1999; a copy of this reference is attached hereto and this reference is cited in the specification at page 11, lines29-31). Because their DNA-binding function and corresponding structures were so well known, the amino acids corresponding to the particular zinc-finger structure and DNA binding function could be and often were removed

from the context of the entire protein to prepare a zinc-finger DNA binding domain peptide (a few of the many examples, include Thukral *et al*., Localization of a minimal binding domain and activation regions in yeast regulatory protein ADR1, *Mol. Cell. Biol*., 9(6):2360-2369, 1989; Hanas *et al*., Internal deletion mutants of Xenopus transcription factor IIIA, *Nucleic Acids Res*., 17(23):9861-70, 1989; Drummond *et al*., "DNA recognition by splicing variants of the Wilms' tumor suppressor, WT1," *Mol. Cell. Biol*., 14(6):3800-3809, 1994; O'Neil *et al*., Functional domain analysis of glass, a zinc-finger-containing transcription factor in *Drosophila*, *Proc. Natl. Acad. Sci. USA*, 92(14):6557-6561, 1995; each of which references is freely available on PubMed at ncbi.nlm.nih.gov/entrez/query.fcgi"). Thus, customary usage of the term zinc-finger DNA binding domain peptide makes clear that such term does not refer to an intact protein containing zinc-finger motifs, but to a relatively small peptide having zinc fingers with which to specifically bind a particular DNA sequence of interest.

As explained in detail above, the specification and customary usage in the relevant art show that the plain meaning of "a zinc finger DNA binding domain peptide" does not encompass full-length EKLF or any portion thereof not limited to the zinc finger domain. Armstrong does not teach a method involving "a zinc finger DNA binding domain peptide"; thus, Armstrong can not and does not anticipate the rejected claims.

In view of the foregoing arguments, Applicant respectfully requests that this rejection be withdrawn.

If the Examiner still has reservations about the plain meaning of "a zinc finger DNA binding domain peptide" as recited in the claims, Applicant would welcome the Examiner's suggestions for mutually acceptable claim language.

CONCLUSION

It is respectfully submitted that the present claims are in a condition for allowance. If it may further issuance of these claims, the Examiner is invited to call the undersigned at the telephone number listed below.

Respectfully submitted,

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Attachment

Klug JMB Reference

[see attached 4 pages]

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JMB

Zinc Finger Peptides for the Regulation of Gene Expression

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Zinc fingers are small DNA-binding peptide motifs that were discovered in this laboratory. These motifs can be used as modular building blocks for the construction of larger protein domains that recognise and bind to specific DNA sequences. Phage display has been used to create a large library of different zinc fingers from which selections were made for binding to a given DNA sequence. From this database there have been elucidated elements of recognition rules that relate the amino acid sequence of a finger to its preferred DNA binding site. Control of gene expression using designed zinc finger peptides has been demonstrated by the specific inhibition of an oncogene mouse cell line and also by switching on genes in expression plasmids. These experiments demonstrate that zinc finger DNA-binding domains can be engineered *de novo* to recognise given DNA sequences. Five to six individual zinc fingers linked together would recognise a DNA sequence 15-18 bp in length, sufficiently long to constitute a rare address in the human genome. By adding functional groups to the engineered DNA-binding domains, e.g. silencing domains, novel transcription factors can be generated to up- or downregulate expression of a target gene. Among potential applications are the repression of oncogene expression and the disruption of the reproductive cycle of virus infection.

Keywords: zinc fingers; gene expression; DNA affinity; phage display

Introduction: protein designs for the recognition of DNA

The selective expression of any one gene is accomplished primarily through the interaction of protein transcription factors with characteristic nucleotide sequences located in the control regions of the gene, which are most commonly located near to, our upstream from, the actual coding region. The binding of a set of such factors, or regulatory proteins, acts as a molecular switch for the activation of the RNA polymerase and other components of the transcriptional machinery, which are common to all genes. The supply of a particular combination of such transcription factors ensures that a gene is switched on at the right place and at the right time.

Eukaryotic DNA-binding transcription factors, like prokaryotic, achieve recognition by having embedded in them a discrete substructure or domain that serves for binding to DNA. Most of the structures identified so far fall into a number of different types, each type containing characteristic amino acid sequences and forming a characteristic three-dimensional structure. The first such structure to be identified was the so-called helix-turn-helix motif (HTH), discovered about ten years ago by X-ray crystallographic studies on certain bacterial regulatory proteins. Since then remarkable progress has been made towards understanding the nature of specific protein-DNA interactions.

A number of types of structural motifs for DNA recognition have been identified on the basis of sequence comparisons, associated with biochemical studies, and the three-dimensional structures have been determined for members of most of these types. Each motif represents a different solution to the problem of designing a piece of protein surface to fit a particular segment of a DNA double helix. DNA-binding domains of proteins have surfaces complementary to DNA and hence a shape, often containing an α-helix, that fits well into the major (or minor) groove of DNA. This is often achieved by an α-helix protruding from the bulk of the whole protein, but presented in different ways in different types of proteins. When the pattern of particular amino acids on the surface of the protein matches the pattern of groups on the surface of the

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DNA double helix, binding takes place through the formation of hydrogen bonds and Van der Waals contacts. One might think of this recognition as a small piece of protein "reading", i.e. interacting with, a short sequence of base-pairs.

A protein reading head could thus recognise a short nucleotide sequence of perhaps three or four base-pairs, but such a short sequence occurs too frequently to be unique and uniquely recognisable. High specificity in the selective control of gene expression requires the recognition of a reasonable length of DNA and Nature has found more than one way of putting together reading heads to achieve this. One design exemplified by several classes of DNA-binding proteins, such as prokaryotic HTH proteins, the hormone receptors and basic leucine zipper (bZIP) proteins, is to combine two monomers so as to bind as a symmetrical dimer with its 2-fold axis coincident with a 2-fold axis of the DNA centred on the palindromic sequence of the DNA binding site. This restricts the generality of the DNA sequence that can be recognised.

The zinc finger motif

In contrast, the zinc finger (Zf) motif first identified in this laboratory in the *Xenopus* transcription factor IIIA suffers no such limitation. Moreover, the zinc finger has a simpler mode of interaction with DNA compared with the other classes of transcription factors.

Each Zf motif forms a small, independently folded zinc-containing mini-domain, used repeatedly in a modular fashion to achieve sequence-specific recognition of DNA. Zf motifs of the TFIIA type have turned up in hundreds of proteins, and they appear to be the most widely used of all types of DNA-binding domains. Indeed, they have been estimated to constitute as much as 1% of human genome. This design may truly be called modular, since the multiply repeated domains all have the same structural framework, but can achieve chemical distinctiveness through variations in certain key amino acid residues.

The zinc finger uses a zinc ion, held by a pair of histidine and a pair of cysteine residues, to stabilise the packing of an antiparallel β-sheet against an α-helix. The crystal structures of zinc finger-DNA complexes show a semiconserved pattern of interactions in which three amino acids from the α-helix (the "recognition helix") contact three adjacent bases (a triplet) in DNA. There are, of course other secondary interactions, but the mode of DNA recognition is principally a one-to-one interaction between amino acids from the recognition helix and bases. Moreover, because the fingers function as independent modules, fingers with different triplet specificities are combined to give specific recognition of longer DNA sequences.

For this reason, the zinc finger motifs are ideal natural building blocks for the *de novo* design of proteins for recognising any given sequence of DNA. Indeed the first protein engineering experiments showed that it is possible to alter rationally the DNA-binding characteristics of individual zinc fingers when one or more of the α-helical positions are varied in a number of proteins. As a large collection of these mutants accumulated, it became possible to propose some rules relating amino acids on the recognition α-helix to corresponding bases in the bound DNA sequence.

Affinity selection from a library of zinc fingers

An alternative to this rational but biased design of proteins with new specificities is the isolation of desirable mutants from a large pool or library. A powerful method of selecting such proteins is the cloning of peptides or protein domains as fusions to the minor coat protein (pIII) of bacteriophage fd. which leads to their expression on the tip of the capsid. Phage displaying the peptides of interest can then be affinity purified and amplified for use in further rounds of selection and for DNA sequencing of the cloned gene. We have applied this technology to the study of zinc finger-DNA interactions, after demonstrating that functional zinc finger proteins could be displayed on the surface of fd phage, and that such engineered phage could be captured on a solid support coated with specific DNA.

Phage display libraries have been created comprising variants of the middle finger from the DNA-binding domain of Zif268 (a mouse transcription factor containing three zinc fingers). DNA of fixed sequence is used to purify phage from this library over several rounds of selection, returning a number of different but related zinc fingers which bind the given DNA. By comparing similarities in the amino acid sequence of functionally equivalent fingers, we can deduce the likely mode of interaction of these fingers with DNA. Remarkably, it appeared that many base contacts occur from three primary positions on the α-helix of the zinc finger, correlating with the implications of the crystal structure of Zif268 bound to DNA. The ability to select or design zinc fingers with desired specificity meant that DNA-binding proteins containing zinc fingers could be made to measure.

Repression of gene expression in a mouse cell line

In the course of the above work, we showed that zinc finger mini-domains could discriminate between closely related DNA triplets, and proposed that they could be linked together to form domains for the specific recognition of longer DNA sequences. One interesting possibility for the use of such protein domains is to target selectively genetic differences in pathogens or transformed cells. Three years ago we reported the first such application, in which we built a protein which recognised a specific DNA sequence both *in vitro* and in *in vivo*.

We created a three finger peptide able to bind site-specifically to a unique nine base-pair region of the p190 *bcr-abl* cDNA: this is a dominant transforming oncogene implicated in Ph^+ acute lymphoblastic leukaemia, and used in cell culture and transgenic mouse models of the disease. The oncogene arises by translocation between chromosomes 9 and 22 and gives rise to the Philadelphia chromosome, which contains a novel DNA sequence at the junction of two exons each from the two parent *bcr* and *abl* genes. The engineered peptide binds to its target site *in vitro* with a K_d of 6×10^{-7} M and can discriminate against like regions of the human genomic *bcr* and *c-abl* genes by factors greater than one order of magnitude.

When fused to a nuclear localisation signal, the peptide accumulates in the nucleus and down regulates levels of p190 *bcr-abl* mRNA. Consequently, stable transformed mouse cells made interleukin-3 independent by the action of the oncogene are found to revert to IL-3 dependence on transient transfection with a vector expressing the peptide. Our construct was engineered to contain a c-myc epitope, which enabled us to follow the localisation of the peptide to the nuclei of the transfected cells. When IL-3 is subsequently withdrawn from cell culture, over 90% of the transfected p190 cells become apopototic within 24 hours. Our experiments were repeated on cells transformed by another related oncogene p210 *bcr-abl*, which served as a control. All transfected p210 cells maintained their IL-3 dependence, and remained intact on entry of the engineered peptide.

Parallel experiments showed that the repression of oncogenic expression by the zinc finger peptide was due to a transcriptional block imposed by the sequence-specific binding of the peptide, which presumably obstructed the path of the RNA polymerase.

Promoter-specific activation

These experiments showed that a zinc finger peptide could be engineered to switch off gene expression *in vivo*. We also carried out experiments on a different cell system (cultured mouse fibroblasts) to show that a gene could also be switched on in a similar way. We used the same nine base sequence as a promoter for a CAT reporter gene contained in a plasmid. The peptide, which recognised the promoter, was fused to a VP16 activation domain and, on transient transfection, stimulated expression of the reporter gene.

Recently we have used this system to ask further whether DNA-binding by a phage-selected zinc finger domain is sufficiently discriminating to allow operations on specific DNA sequences *in vivo*. We used the CAT transactivation assay to investigate the ability of a model phage-selected DNA-binding domain to discriminate its target from a set of closely related promoter sequences. We found that this was possible with a high degree of specificity, but only when the intracellular concentration of zinc finger transcription factor was optimised. The reason is that, while the affinity of such phage selected DNA-binding domains is quite high, usually falling in the nanomolar range for a three-finger peptide, the observed discrimination against closely related DNA sequences, by a two- to tenfold increase in the apparent K_d per point mutation, is rather modest. These results warrant the use of phage-selected DNA-binding domains to direct *in vivo* DNA manipulation, in particular the control of gene expression, but invoke the need for controlled delivery of the DNA-binding domain in order to achieve specific effects.

Improving zinc finger specificity

Other recent work has focused on improving the specificity of recognition by zinc fingers of the DNA target. While the main source of specificity lies in the amino acids at positions −1, 3 and 6 of the recognition α-helix of a zinc finger for successive bases lying on one strand of a DNA triplet, we have found that a "cross-strand" interaction from helical position 2 to the neighbouring base-pair on the adjacent triplet can influence the specificity. Therefore it has been necessary to revise the simple model that zinc fingers are essentially independent modules that bind three base-pair subsites to a model that considers synergy between adjacent zinc fingers. In this revised model, Zif268-like zinc fingers potentially bind four base-pair overlapping subsites. We have now re-designed our method of phage library construction to take account of this refinement.

Concluding remarks

We have shown that a DNA-binding protein can be designed *de novo* to recognise a specific DNA sequence *in vitro* where, directed to the nucleus by an appended localisation signal, it can bind its target sequence in chromosomal DNA, causing a specific inhibition of transcription. The design of peptides able to bind oncogenes has obvious implications for cancer research, but our primary aim was to prove the principle of protein design and to assess the feasibility of *in vivo* binding to chromosomal DNA in available model systems. The use of a blocking agent, in this case to target intragenic sequences, is reminiscent of antisense oligonucleotide or ribozyme-based approaches to inhibiting the expression of selected genes. Like antisense oligonucleotides, zinc finger DNA-binding proteins can bind to various unique regions outside the coding region of a gene. But in contrast to the use of nucleic acids, proteins can direct gene expression by both up or down-regulating the initiation of transcription when designed to bind promoter regions and fused to activation or repression domains.

By acting directly on any DNA, and by allowing fusion to a variety of protein effectors, tailored site-

specific DNA-binding proteins have the potential to control expression of specific genes and to further the possibility of manipulating the genetic material itself, in medicine and research. The problem which remains, as in all methods of gene therapy, is the development of effective delivery systems. There is every hope that this will be achieved in the near future and that such systems will be used to convey the tailored proteins to their DNA targets. To date, the zinc finger motif remains the best natural design for targeting DNA, since it takes advantage of the modular principle which offers a very large number of combinatorial possibilities for sequence specific recognition of DNA.

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